510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k042071

B. Purpose for Submission:
New device

C. Analyte:
Lactoferrin

D. Type of Test:
Quantitative ELISA

E. Applicant:
TechLab®, Inc.

F. Proprietary and Established Names:
IBD-SCAN® Test

G. Regulatory Information:

1. Regulation section:
   21CFR§ 866.5570, Lactoferrin, Immunological Test System
2. Classification:
   Class II
3. Product Code:
   DEG, Lactoferrin, Antigen, Antiserum, Control
4. Panel:
   82 Immunology

H. Intended Use:

1. Intended use(s):
   The IBD-SCAN® test is a quantitative ELISA for measuring concentrations of fecal lactoferrin, a marker of fecal leukocytes. An elevated level is an indicator of intestinal inflammation. The test can be used as an *in vitro* diagnostic aid to distinguish patients with active inflammatory bowel disease (IBD) from those with noninflammatory irritable bowel syndrome (IBS).
2. Indication(s) for use:
   Same as above
3. Special condition for use statement(s):
   This device is for prescription use only.
4. Special instrument Requirements:
   Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter

I. Device Description:

The IBD-SCAN® test is a quantitative ELISA for determining levels of lactoferrin in human fecal specimens. The assay components include the following:

- 10X Diluent (10X concentrate of a buffered protein solution containing 0.2% thimerosal). The 1X Diluent is used as the negative control.
- Conjugate (rabbit polyclonal antibody specific for human lactoferrin conjugated to horseradish peroxidase).

- Substrate (solution containing tetramethylbenzidine substrate and peroxide)
- Standards (5 levels of human lactoferrin in a buffered protein solution)
- Positive Control (10 µg/mL; human lactoferrin in buffered protein solution)
- Wash Buffer Concentrate (20X containing phosphate buffered saline)
- Stop Solution (0.6N sulfuric acid)
- Microassay Plate (12 strips, 8 wells per strip coated with purified polyclonal antibody specific for lactoferrin)

J. Substantial Equivalence Information:

1. Predicate device name(s):
   IBD-CHEK®
2. Predicate K number(s):
   k011396
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	IBD-SCAN	**IBD-CHEK**
Intended Use	Detection of lactoferrin, a marker of fecal leukocytes	Same
Sample matrix	Human fecal specimen	Same
Differences		
Item	**Device**	**Predicate**
Assay principle	Quantitative ELISA	Qualitative ELISA
Standards	Primary and Secondary standards for assigning quantity of lactoferrin in the device	None provided

K. Standard/Guidance Document Referenced (if applicable):
None referenced

L. Test Principle:
The IBD-SCAN® test uses antibodies to human lactoferrin. The microassay wells supplied with the kit contain immobilized polyclonal antibody against lactoferrin. The detecting antibody consists of polyclonal antibody conjugated to horseradish peroxidase. Standards and serial dilutions of fecal specimens are transferred to the microassay wells. If detectable levels of lactoferrin are present in the specimen, the lactoferrin binds to the immobilized antibody. After incubation, the wells are washed and the antibody conjugate is added. The conjugate binds to the lactoferrin bound during the first incubation phase. A second series of wash steps removes any unbound material. Following the addition of substrate, a color is detected due to the enzyme-antibody antigen complexes that form in the presence of lactoferrin. Lactoferrin standards ranging from 6.25 to 100 ng/mL are used to generate a standard curve. By plotting absorbance values versus lactoferrin concentrations, the lactoferrin concentration in a test sample can be determined.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Intra-assay precision

The intra-assay precision of the IBD-SCAN ™ test was determined by analyzing reactions among nine fecal specimens. Five specimens had elevated lactoferrin and four specimens with baseline lactoferrin. Each specimen was tested in quadruplicate. The %CV ranged from 0 to 16% with a mean value of 10.9%.

Inter-assay precision

The inter-assay precision was determined by analyzing nine fecal specimens over a 3-day period. Five specimens had elevated lactoferrin and four specimens with baseline lactoferrin. Each specimen was tested in quadruplicate. The %CV ranged from 0 to 47.7% with a mean value of 18.6%.

b. Linearity/assay reportable range:

Two standard curves were generated in each of three separate lots of kits. The results with serially diluted lactoferrin in the three lots showed linearity up to100 ng/mL. The following equations were obtained:

Standard curve 1, $y = 0.0079x - 0.007$, $R^2 = 0.9991$

Standard curve 2, $y = 0.0057x + 0.0074$, $R^2 = 0.9973$

Standard curve 3, $y = 0.0084x - 0.009$, $R^2 = 0.9951$

c. Traceability (controls, calibrators, or method):

Recombinant human lactoferrin obtained from Ventria Bioscience is available through two sources: Sigma Chemical Company and USB Corporation. The recombinant human lactoferrin as the primary standard is used for the quality control of the Secondary Standard, native human lactoferrin. Chemical characteristics and specifications for the reference materials are provided.

Comparative analyses of the immunoreactivity of the primary and secondary lactoferrin standards were performed. Analyses were done based on the protein concentrations and the immunoreactivity determined by the IBD-SCAN™ test. The protein concentration of both standards was prepared based on the results listed on the corresponding certificate of analysis. The immunoreactivity was determined by the IBD-SCAN™ test according to the package insert using serial two-fold dilutions of the purified recombinant or human lactoferrin in the kit diluent. Best fit curve analysis of the results (as measured by A_{450} values) demonstrated correlation coefficient (R^2) values of 0.997 and 0.996 for recombinant and native lactoferrin respectively.

d. Detection limit:

The lower limit of detection was determined from the mean absorbance values for negative controls consisting of sample diluent (referred to as "blank") and standard concentrations of lactoferrin (referred to as "standard") using 2 ng/mL and 4 ng/mL concentration. Two assays were done. Assay 1 contained 10 standards of 2 ng/mL of lactoferrin and Assay 2 contained 10 standards of 4 ng/mL of lactoferrin. Both assays used 10 blanks. The lower limit of quantitation was determined by the lactoferrin concentration that generated a mean absorbance value that did not overlap the mean value of the blanks. Significant difference was determined using a two-tailed T-test with a p-value of <0.002. The lower limit of detection was 4 ng/mL of lactoferrin.

e. Analytical specificity:

A variety of normal microorganisms typically found in the human intestinal tract were evaluated for reactivity in the IBD-SCAN™ test. In addition, a variety of pathogenic organisms that cause intestinal disease were evaluated. The pathogens included in the study cause inflammatory diarrhea or diarrhea without significant levels of inflammation.

For the analysis, each organism was grown in appropriate media and the turbidity of each culture was determined, based on McFarland standards, to be at least 10^8 per mL. Aliquots (0.1 mL of each culture) of each organism were assayed using the device. None of the organisms reacted in the IBD-SCAN™ test

f. Assay cut-off:

The IBD-SCAN™ test utilizes a cut-off of ≥ 7.25 ug/mL in feces for defining elevated lactoferrin which was determined as the mean lactoferrin level of healthy persons plus 2 standard deviations. This cut-off was validated using comparison studies with the IBD-CHEK test and clinical assessments with disease activity.

2. Comparison studies:

a. Method comparison with predicate device:

Study #1 was performed at two IBD centers. All of the patients involved in the study were clinically documented on-site with ulcerative colitis, Crohn's disease, or irritable bowel syndrome. Also included were specimens from healthy persons. Each enrolled patient was assessed by the attending physician to determine the following: Harvey Bradshaw Activity Index (HBAI) for Crohns disease and Modified Harvey Bradshaw Activity Index for ulcerative colitis or IBS symptom questionnaire. The Harvey Bradshaw Activity Index is a measure of disease activity in IBD whereby a scoring method based on symptoms is calculated by a physician. The use of a clinically based index for disease activity is the gold standard for studies involving IBD.

A total of 149 IBD patients were enrolled and comprised of 51.7%males and 48.3% females. The approximate 1:1 ratio is similar to male: female ratio typically observed in IBD population. The ages ranged from 3 to 78 years. Of the total IBD group, 32 (21.5%) were ≤16 years. The IBS patient group had 31patients with 19.3%males and 80.7% females and an age range of 19 to 78 years. This ratio of males to females is similar to the 1 to 7 male: female ratio typically reported in IBS patient populations. Fifty-five healthy persons were also enrolled with approximately 1:1 male to female and an age range of <1 to 79 years. Statistical analysis of the IBD-SCAN test results compared to the IBD-CHEK test for detecting intestinal inflammation showed the following results:

N = 235	IBD-CHEK test positive	IBD-CHEK test negative
IBD-SCAN test elevated	105	10
IBD-SCAN test baseline	7	113

Agreement for positives	93.8%
Agreement for negatives	91.9%
Percent Agreement	92.8%

Study #2 was to evaluate the IBD-SCAN™ test in patients with inflammatory bowel disease or irritable syndrome. A total of 93 IBD patients and 2 IBS patients were enrolled. The IBD patients comprised of 32%males and 68% females with ages ranged from 4 to 51 years. Twenty-five percent of the IBD patients (23) were ≤16 years. The 2 IBS patients were 50 and 59 year old females. Statistical analysis of the IBD-SCAN test results compared to the IBD-CHEK test for detecting intestinal inflammation.

N = 95	IBD-CHEK test positive	IBD-CHEK test negative
IBD-SCAN test elevated	57	6
IBD-SCAN test Baseline	1	32

Agreement for positives	98.3%
Agreement for negatives	83.8%
Percent Agreement	92.6%

b. Matrix comparison:

Human feces are the only recommended matrix.

3. Clinical studies:

a. *Clinical sensitivity:*
The same studies mentioned in the Method Comparison were used to assess sensitivity and specificity of the assay. An additional study using pediatric specimens was also performed. Study #1 was to evaluate the IBD-SCAN™ test in patients with inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS) and in healthy persons. Statistical evaluation using the IBD-SCAN test to distinguish active inflammatory bowel disease from irritable bowel syndrome/healthy persons showed the following results

N=178	**Active IBD based on HBAI assessment ≥4**	**IBS (31)/Health persons (55) based on clinical self assessment**
IBD-SCAN test elevated	75	3
IBD-SCAN test baseline	17	83

Clinical sensitivity 81.5%
Clinical specificity 96.5%

Statistical evaluation using the IBD-SCAN test to distinguish ulcerative colitis from irritable bowel syndrome/healthy persons

N=127	**Active IBD based on HBAI assessment ≥4**	**IBS (31)/Health persons (55) based on clinical self assessment**
IBD-SCAN test elevated	37	3
IBD-SCAN test baseline	4	83

Clinical sensitivity 90.2%
Clinical specificity 96.5%

Study #2 was to evaluate the IBD-SCAN™ test in patients with inflammatory bowel disease or irritable syndrome. Statistical analysis of the IBD-SCAN test results to distinguish active ulcerative colitis from active irritable bowel syndrome and inactive UC as defined by clinical histories and IBD-CHECK results.

N=25	**Active ulcerative colitis4**	**Inactive ulcerative colitis**
IBD-SCAN test elevated	12	4
IBD-SCAN test baseline	0	9

Clinical sensitivity 100%
Clinical specificity 69.2%

Statistical analysis of the IBD-SCAN test results to distinguish active Crohn's disease from active irritable syndrome and inactive CD as defined by clinical histories and IBD-CHEK results.

N=72	Active Crohn's disease	Inactive CD and IBS
IBD-SCAN test elevated	45	2
IBD-SCAN test baseline	1	24

Clinical sensitivity 97.8%
Clinical specificity 92.3%

Study #3 was to evaluate the IBD-SCAN™ test in pediatric patients with IBD and noninflammatory gastrointestinal illnesses. Statistical evaluation using the IBD-SCAN test to distinguish active inflammatory bowel disease from irritable bowel syndrome/other noninflammatory intestinal illnesses

N=51	Active IBD based on Physician's assessment	IBS/Other noninflammatory conditions-based on Physician's assessment
IBD-SCAN test elevated	40	0
IBD-SCAN test baseline	0	11

Sensitivity 100%
Specificity 100%

Statistical evaluation using the IBD-SCAN test to distinguish active ulcerative colitis from irritable bowel syndrome/other noninflammatory intestinal illnesses

N=35	Active ulcerative colitis based on Physician's assessment	IBS/Other noninflammatory conditions-based on Physician's assessment
IBD-SCAN test elevated	24	0
IBD-SCAN test baseline	0	11

Sensitivity 100%
Specificity 100%

b. Clinical specificity:
See above.

c. Other clinical supportive data (when a and b are not applicable):
Not applicable.

4. Clinical cut-off:
   Not applicable
5. Expected values/Reference range:
   See Assay cut-off.

N. Conclusion:

The submitted material in this premarket notification is complete and supports a substantial equivalence decision.